FECAL BILE ACIDS OF BLACK-FOOTED FERRETS

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ABSTRACT.—Fecal bile acid characteristics have been used to identify scats to species of origin. Fecal bile acids in scats from 20 known black-footed ferrets (*Mustela nigripes*), 7 other known small carnivores, and 72 of unknown origin were analyzed to determine if this procedure could be used as a tool to verify ferret presence in an area. Seventeen ferret scats were suitable for analysis and had a mean fecal bile acid index of 156 ± 9. This was significantly different from mean indices for the other carnivores; however, substantial overlap among confidence intervals occurred for badgers, kit foxes, and especially long-tailed weasels. We conclude this method is not useful for making positive identifications of individual ferret scats and suggest that we may be able to definitively identify individual scats with reasonable confidence by using gas-liquid chromatography.

A major research goal of the Meeteetse, Wyoming, black-footed ferret (*Mustela nigripes*) (BFF) studies is development of survey techniques (Clark 1984). From 1981 to 1984, 92 scats, 20 of known BFF origin and 72 of unknown origin but similar in size, shape, and color to known BFF scats, were collected (BFF scats pictured on p. 20 in Clark et al. *Handbook of methods*, 1984). Fecal bile acid analyses have been used to identify scats (Major et al. 1980, Johnson et al. 1984). Analysis may be performed by thin-layer (TLC) or gas-liquid (GLC) chromatography (Johnson et al. 1984). Although the latter method is more quantitative, it is also much more time consuming and expensive than TLC and requires additional training. Costs for routine management applications would probably be prohibitive for most government fish and wildlife agencies, especially if analyses are needed for a large collection of scats. TLC can be performed in less time, and several samples can be analyzed at the same time. Initial equipment expense for TLC is about 20% of cost for GLC. The purpose of this study was to determine if thin-layer chromatographic analyses of fecal bile acids could be used as a means to positively identify scats from BFFs and thereby provide a new tool to determine BFF presence in an area.

METHODS

Scats from 20 BFFs were obtained from live-trapped specimens; they were collected along tracks of ferrets in snow (Clark et al. *Handbook of methods*, 1984; Clark et al. *Seasonality of black-footed ferret diggings*, 1984) or after field observers saw animals defecate. Another 72 scats each were collected from uncertain identity from the same area where field personnel collected the known BFF scats. To cover the range of size of the unidentified scats, 5 or 10 known scats each were collected from seven additional carnivore species that may frequent prairie dog (*Cynomys* sp.) colonies (Clark et al. 1982) 1979–1984: badgers (*Taxidea taxus*), long-tailed weasels (*Mustela frenata*), mink (*M. vison*), kit fox (*Vulpes macrotis*), striped skunk (*Mephitis mephitis*), red fox (*Vulpes vulpes*), and gray fox (*Urocyon cinereoargenteus*).

All scats were analyzed according to the thin-layer chromatographic method (TLC) described by Major et al. (1980). Visualization of steroid bands on TLC plates was accomplished by spraying with 8–hydroxy-1,3,6–pyrenetrisulfonic acid trisodium salt (5 mg in 100 ml of methanol). This reagent was used in lieu of that used by Major et al. (1980) because it does not destroy the steroids. After visualization, locations of all steroid bands were recorded relative to the solvent front (rf). Only bands that occurred between rf values of 15% and 75% of the solvent front were consid-

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ered to be fecal bile acids. Cholic and lithocholic acids are present in most species scats and usually travel at least 15% and 75% of the solvent front, respectively, using this technique (Major et al. 1980). Bile acids can be eluted from this silica gel and used for future GLC analyses.

Because there is variation in fecal bile acid concentration, we categorized scats that had less than three distinct bile acid bands as unidentifiable. This decision was justified because scats from no species previously described other than mountain lion (*Felis concolor*) have had fewer than three detectable fecal bile acids on TLC plates (Major et al. 1980, Johnson et al. 1981, Johnson and Aldred 1981, Johnson et al. 1984). Fresh scats from known specimens do not usually produce low quantities of fecal bile acids. However, weathered scats may, because bile acids are highly soluble in water. An average fecal bile acid index was obtained for each species by summing rf values for all bands in each scat and averaging among scats. Statistical analyses were performed by comparison of mean indices among species. Data herein are means and standard errors.

RESULTS

Comparison of Known Scats

The number of bile acid bands and index means varied among the eight carnivore species. Of 20 BFF scats, three contained fewer than three bile acid bands, seven had three bands, three had four bands, six had five bands, and one had six bands on TLC plates. The mean fecal bile acid index for BFF scats with three or more bands was 156 + 9 (Table 1).

For the other seven carnivores, the number of TLC bands ranged from three to seven. Striped skunk, gray fox, and red fox scats never produced more than three bands; index means were 96 ± 1, 93 ± 3, 94 ± 2, respectively, and were significantly smaller than the BFF index ($P < .05$). The 80% confidence intervals for these species compared to those for BFFs suggested that their scats would probably not be confused with BFF scats by TLC analysis (Table 1). The mean index for mink scats (78 ± 4), which had three or four bands, was also significantly smaller than the mean for BFFs ($P < .05$), and 80% confidence intervals did not overlap.

Mean fecal bile acid indices for badgers (179 ± 22), long-tailed weasels (197 ± 16), and kit fox (243 ± 17) were significantly larger than the mean for BFF scats at the 0.05 level of probability for type I error. However, there was substantial overlap among confidence intervals between each of these species and BFF scats.

Regarding this overlap, less than 1% of BFF scats having an index less than 153 would be confused with kit fox scats, and less than 5% would be confused with long-tailed weasel scats. For BFF scats with an index less than 163, less than 10% would be confused with long-tailed weasel scats. Variation in fecal bile acid indices from badger scats was so large that they could not be distinguished from BFF indices. Badger scats can often be differentiated by size from BFF scats; however, size overlap does sometimes occur, which is a problem for visual analysis.

Ten (50%) of the BFF scats produced at least three bands on TLC plates and produced indices less than 163; nine (45%) of these were less than 153. Only four (20%) BFF scats produced fecal bile acid indices that were less than 163 and within the 99% confidence interval for BFFs. Three of these scats produced indices that were less than 153.

About 35% of the BFF indices were too large to be distinguished from indices from kit fox or long-tailed weasel scats using TLC analysis. We estimate a 15% probability of identifying a BFF scat with 99% confidence that it is not from a kit fox. A 15% probability exists of identifying that a BFF scat is not from a long-tailed weasel with 95% confidence; or only a 20% probability of identification with 90% confidence.

In addition to the problem of misclassifying BFF scats, long-tailed weasel scats can be misclassified as BFF scats. No kit fox scats produced indices within the 80% or 99% confidence intervals for BFF indices, so this would be an improbable source of error. However, 40% of long-tailed weasel scats produced indices that were within the 99% and 80% confidence intervals for BFF scats, thus creating a significant problem with this analysis. The other 60% (three of five) long-tailed

TABLE 1. Mean fecal bile acid indices and confidence intervals for scats from seven carnivore species with at least three detected steroid bands between rf 15–70 on 20-cm silica gel G TLC plates.

Species	N	Mean ± SE	Confidence intervals 99%	95%	90%	80%
Black-footed ferret	17	156 ± 9	129–183	137–176	140–173	144–169
Badger	5	179 ± 22	69–274	109–233	123–219	131–211
Long-tailed weasel	5	197 ± 16	124–271	153–242	163–232	173–222
Kit fox	5	242 ± 17	166–321	196–290	207–280	218–269
Mink	10	78 ± 4	65– 91	69– 87	71– 85	72– 84
Striped skunk	10	96 ± 1	93– 99	94– 98	94– 98	95– 97
Gray fox	10	93 ± 3	83–103	86–100	88– 98	89– 97
Red fox	10	94 ± 2	87–101	89– 99	90– 98	91– 97

weasel scats produced indices larger than those within the 99% confidence interval for BFFs.

Identifying Unknown Scats

For the 72 unidentified scats from areas known to have BFFs, 14 produced indices less than 163, and 8 of these were within the 99% confidence intervals for BFFs and long-tailed weasels. Only 2 of the 8 scats produced indices below 153. We are confident that these scats were from BFFs because they were much smaller than most badger scats, were associated with apparent BFF sign, and had indices (both 138) outside the 99% confidence intervals for species other than long-tailed weasels. They were probably not from long-tailed weasels because the smallest weasel index we obtained was 158. We recognize that this is the weakest point in our data because of the small number of known long-tailed weasel scats examined.

There were 12 other scats that produced indices within the 99% confidence interval for BFFs, but these indices were higher than the mean and could not be distinguished from indices for kit fox, long-tailed weasel, or badger. This analysis suggests that to be reasonably certain that BFFs are present in any area, some of the scats must produce fecal bile acid indices between 129 and 153. Only 15% (3 of 20) of bona fide BFF scats produced indices in this range. Therefore, there is a relatively large probability of not detecting BFF presence when few scats are examined.

Although we estimated that about 36% (26/72) of the unidentified scats were likely BFF scats, only about 3% (two/72) of these scats were within the 95% confidence interval for BFFs. Assuming that no long-tailed weasel scats will produce a fecal bile acid index less than 153, then a rough estimate for the number of the 72 scats that actually were BFF scats is 13 (2 ÷ 0.15).

DISCUSSION

Although sample sizes were relatively small, this analysis suggests that the TLC method is not useful for making positive identifications of individual BFF scats. Because of variation among fecal bile acid indices for BFFs and other carnivores, an individual scat could not be positively identified even though mean fecal bile acid indices were significantly different among the species. Differentiation of long-tailed weasel, small badger, and BFF scats is the most significant problem identified by our analysis.

Because about 15% of known BFF scats seemed to produce index values distinctly different from those produced by other carnivores, it may be possible to establish a high probability of BFF presence using indices from a relatively large collection of scats. However, whether or not this can be done depends on obtaining a better description of variation in indices produced by scats of other species, particularly long-tailed weasels and to some extent badgers. More known scats must be analyzed to improve our data base.

The differences found in fecal bile index means suggests that a more definitive type of analysis might provide a bona fide technique that can be used to identify individual scats with reasonable confidence. Recently Johnson et al. (1984) demonstrated that gas-liquid chromatography (GLC) provided a more definitive distinction between fecal bile acids of bobcat (*Felis rufus*) and mountain lion than

TLC. We suggest that additional effort be expended to obtain gas-liquid chromatographs as a method for identifying BFF scats. Although GLC costs are very high compared to TLC, new developments in equipment and methods will probably reduce costs substantially in the future.

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